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# Full-Scale Anaerobic Bioremediation of Trinitrotoluene (TNT) Contaminated Soil

## A US EPA SITE Program Demonstration

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## ABSTRACT

An anaerobic bioremediation process for the degradation of nitroaromatic compounds in soil was demonstrated. This *ex situ* process was demonstrated full-scale at a 2,4,6-trinitrotoluene (TNT)-contaminated site near Weldon Spring, MO. A bioreactor was loaded with approx 23 $m^3$ of TNT-contaminated soil in the form of a 50:50 soil: water slurry. This slurry was augmented with a starchy carbon source (1–2% w/v) and buffered with phosphate to near-neutral pH. Indigenous soil bacteria utilized the oxygen, making the slurry anaerobic within 1–2 d. Anaerobes then degraded the TNT (3000 mg/kg) in approx 11 wk. A relatively long treatment time for the bioremediation of the TNT-contaminated soil was necessary, possibly because of the cool ambient temperatures, high clay content of the soil, high level of contamination, and high level of recalcitrance of TNT in soils.

**Index Entries:** Trinitrotoluene; dinoseb; degradation; bioremediation; soil.

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## INTRODUCTION

Hazardous nitroaromatic compounds have been known to contaminate many soil environments (*1*). Munitions compounds, including TNT and dinitrotoluenes (DNTs), exist as waste products from Department of Defense (DOD) facilities and usually persist in soil for many years (*2,3*). Nitroaromatic pesticides, such as 2-*sec*-butyl-4,6-dinitrophenol (dinoseb), are also known to contaminate soil and ground water (*2*) and are structurally similar to nitroaromatic explosives. This pesticide is now banned by the US Environmental Protection Agency (EPA) because of its toxic and teratogenic nature. Methods to remediate these widespread, existing contaminated soils economically are needed. The US Army has worked on a method to compost these explosive-contaminated soils (*4*). By the addition of a bulking agent, composting was able to remove TNT. However, the fate of the parent molecule remains unknown, and it may be that the parent molecule has only been polymerized or become reduced and bound to the humic material.

We have examined the treatability of soils contaminated with nitroaromatic compounds using bacteria (*2,3*). TNT and dinoseb were removed from contaminated soils by an anaerobic consortium of indigenous soil bacteria. These soils were pretreated by the addition of a starchy potato-processing byproduct and were buffered with phosphate-based buffer. Oxygen was consumed quickly, lowering redox potentials to $< -200$ mV within a few days. After anaerobiosis was established, an anaerobic consortium proliferated and reduced concentrations of TNT and dinoseb from 120 and 80 ppm, respectively, to nondetectable levels (*2,3*). By monitoring the concentrations of parent molecules and intermediates of degradation, and by use of $^{14}$C-labeled TNT and dinoseb as substrates, it was shown that both of these nitroaromatics were transformed to nonaromatic products under the growth conditions used. The J. R. Simplot Co., Boise, ID, a sponsor of this research, has patented this technology as the SIMPLOT™ process (*5,6*).

The EPA's Superfund Innovative Technology Evaluation (SITE) program previously conducted a similar demonstration of this technology, bioremediating 40 yd$^3$ of dinoseb-contaminated soil (*2*). This demonstration took place at Bowers Field in Ellensburg, WA, during the summer of 1993. Dinoseb-contaminated soils were excavated, pretreated, and bioremediated in a 60,000-L bioreactor. Before treatment, the soil had an average dinoseb concentration of 25 mg/kg, and this was reduced to below the analytical detection limit (0.15 mg/kg) in $<25$ d (*5*).

The purpose of this article is to present data obtained in another SITE demonstration conducted by the J. R. Simplot Co. for the bioremediation of TNT from soil. This demonstration was performed at the Weldon Spring Ordnance Works (WSOW) site in Weldon Springs, MO, approx 30 miles west of St. Louis. WSOW formerly produced TNT and DNT in large quan-

tities (over 350,000 t) from 1941 to 1945 (*6*). High levels of these nitroaromatics have persisted in this soil. Testing the biomediation method on this soil was done in a preliminary effort to remove the contaminants as required by the EPA.

## MATERIALS AND METHODS

### Soil

Twenty-three cubic meters of contaminated soil were excavated near the vicinity of an old TNT production line. The soil was screened to 1/2 in. using sifter screens and was found to have a high clay content (18.8% by Bouyoucas hydrometer method). Large rocks and clods of soil were washed with hot water in a modified cement mixer, and the waste water was saved for use in the treatment process. The sifted soil was amended with starch to 1–2% (w/v) using a high-shear pug mill mixer and then added to the bioreactor.

### Bioreactor

A custom-designed, 75,000-L, portable steel bioreactor (12.3 m long, 2.5 m wide, and 2.6 m tall) with three top-mounted, low-speed, high-shear mixers was used to bioremediate this soil. This bioreactor was trailer-mounted, resembling a small railroad car, and was open on the top. The bioreactor held about 40,000 L of buffered water and 40 $m^3$ of soil.

### Process

The SIMPLOT™ process involves the homogenization of 50/50 (w/v) soil, 1–2% starch, and a phosphate buffer mixture (a mono- and dipotassium phosphate mixture, pH 7.3) in a bioreactor. To eliminate unmixed "hot pockets" of TNT, a double-diaphragm pump was used weekly to recirculate the aqueous phase and hydrolance the soil in a manner that mixed the slurry vigorously, but did not introduce much oxygen.

Waste water from the rock washing is included in the mixture. Earlier, during the dinoseb bioremediation demonstration, stirring of the dinoseb-containing soil was accomplished with high-shear electric mixers. The TNT-contaminated soil was much more difficult to stir because of the high clay content of the soil. Therefore, the mixers were replaced by hydrolancing the soil throughout the bioremediation process. When the outside temperature fell to near freezing, three (3-kw) immersion heaters were installed to keep the reactor temperature near 25°C. Ninety-eight percent sulfuric acid was added (200–1500 mL) at times in efforts to maintain the pH below 7.6. This was accomplished using vigorous mixing to dilute the acid quickly, thus preventing cell death.

### Analytical

pH, temperature, and redox were continually monitored by computer-coupled instruments installed on the bioreactor. Soil (100–300 g) and aqueous (100–200 mL) samples were gathered daily from five areas at various depths throughout the bioreactor. TNT concentrations were determined in the field using EnSys field test kits (EnSys, Research Triangle Park, NC). Levels of contamination for TNT and 4-amino-2,6-dinitrotoluene (4A26DNT) were confirmed, using EPA method 8330, by an independent analytical company (Science Applications International Corp., Idaho Falls, ID). Final samples were also analyzed for the following intermediates: 2,4-diamino-6-nitrotoluene (24DA6NT), 2,6-diamino-4-nitrotoluene 26DA6NT), 2,4-6-triaminotoluene (TAT), methylphloroglucinol (MPG, trihydroxytoluene), and *p*-cresol (EPA HPLC method 8330). The data reported here are considered accurate, but preliminary, and remain to be formally approved as "final" by the US EPA.

## RESULTS

Bioremediation was initiated on September 19, 1993, and the arbitrary termination date was February 14, 1994. The pH values of the slurry mixture were recorded at daily intervals and are plotted against time as shown in Fig. 1. Several times during the bioremediation process, the pH of the slurry mixture rose close to 7.5, and 98% sulfuric acid was then added in order to maintain the pH near neutral.

Figure 2 shows the redox potential of the bioreactor mixture during the bioremediation process. The redox potential was initially $>0$ mV, and then remained below 0 mV throughout the remainder of the process. The apparent sudden increase in redox potential between days 144 and 149 was an artifact owing to malfunctions of the redox probe.

The temperature of the slurry mixture was initially uncontrolled and was affected by the ambient temperature. The immersion heaters were added to the bioreactor when the soil slurry temperature dropped to below 5°C. Bioreactor temperature was then maintained above 15°C (Fig. 3).

The WSOW soil that entered the reactor initially contained an average of 3000 mg/kg of TNT (dry wt). This was bioremediated to $<1$ mg/kg during the 5-mo long process. Figure 4 shows the decline in TNT concentration (average of all samples collected). The rise and decline with time of the first intermediate, 4A26DNT, is also plotted in Fig. 5.

Analyses of final samples showed that TNT and 4A26DNT had been removed by the microbial consortium. However, there were still small amounts ($\leq 50$ ppm) of 24DA6NT. In addition, some, but not all, samples contained detactable levels of *p*-cresol at times during the bioremediation process (T. Jackson, personal communication). TAT, MPG, and 26DA4NT were not detected.

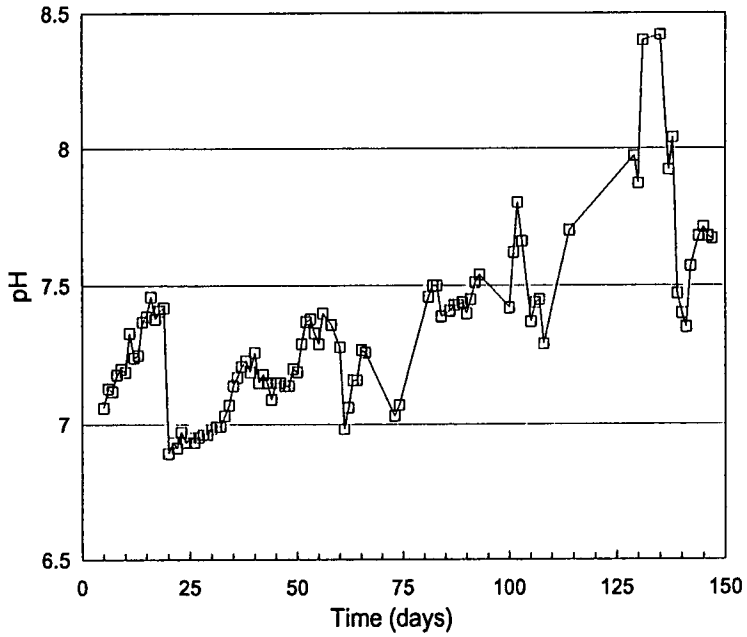


Fig. 1. pH values vs time for the bioreactor slurry of WSOW soil. Values were obtained daily using a computer-coupled pH probe.

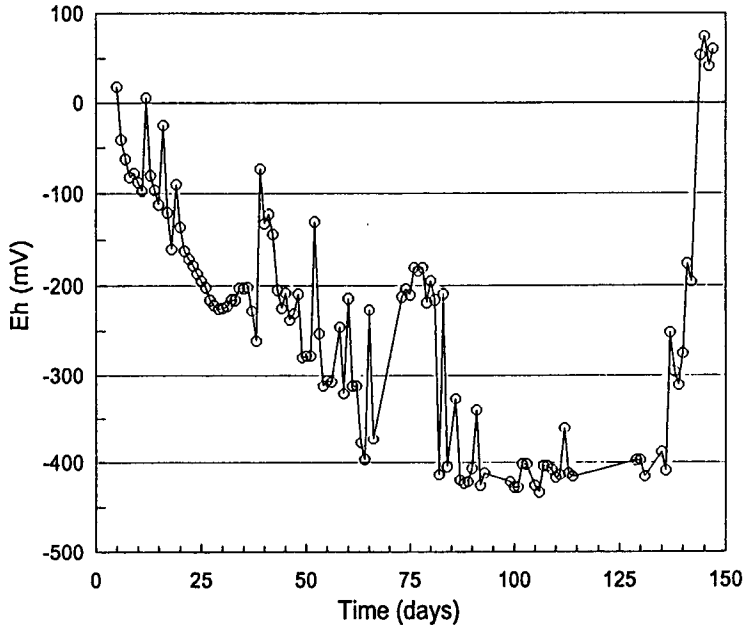


Fig. 2. Redox potential values vs time for the bioreactor slurry of WSOW soil. Readings were obtained daily using a computer-coupled redox probe.

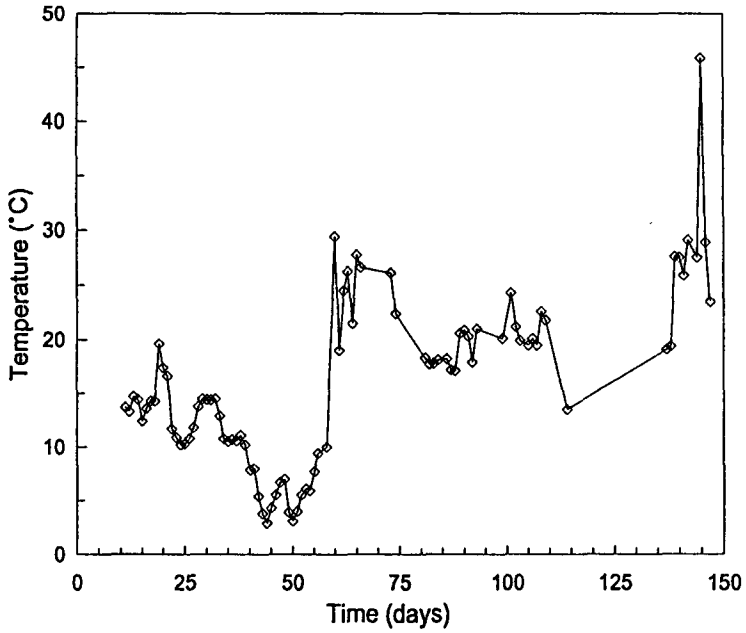


Fig. 3. Temperature of the bioreactor slurry mixture vs time. Readings were obtained daily using a computer-coupled temperature probe.

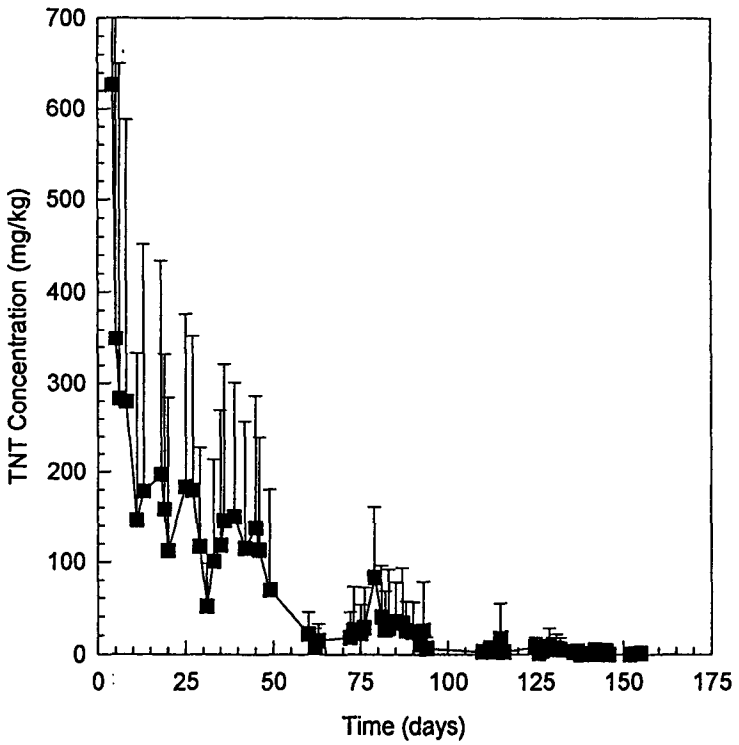


Fig. 4. Time-course analyses of TNT concentration during the anaerobic remediation of WSOW TNT-contaminated soil. Positive error bars = 1 SD.

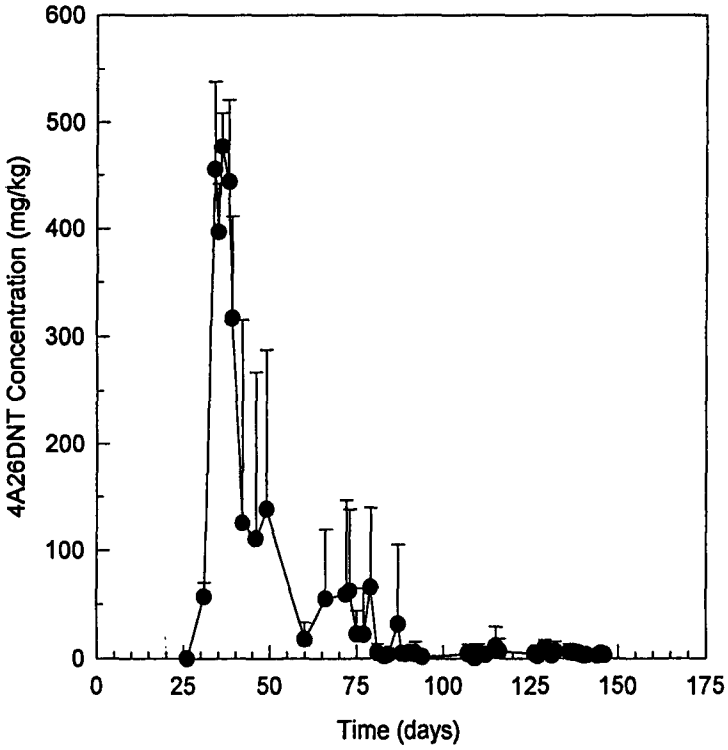


Fig. 5. Time-course analyses of 4A26DNT concentration during the anaerobic remediation of WSOW TNT-contaminated soil. Positive error bars = 1 SD.

## DISCUSSION

This SITE demonstration completed the EPA's second effort to test Simplot's bioremediation technique on nitroaromatic compounds. Dinoseb was also successfully bioremediated from 40 $m^3$ of soil. This soil contained 24 mg/kg of dinoseb, and anaerobes degraded it within 2 wk of incubation using the same bioreactor that was used for this demonstration.

For this TNT demonstration, some adjustments were necessary to maintain optimal biological activity. Sulfuric acid was used to lower the pH as the slurry mixture became alkaline. During the degradation of parent TNT, it is theorized that ammonia is continually released into the supernatant. Although nitrogen levels, as nitrate, nitrite, or ammonia, were not monitored throughout the bioremediation process, it is thought that the cleavage of amino groups from the TNT intermediates could be responsible for the increases in pH. The pH was tracked carefully to ensure that it did not rise above 7.6. Above this threshold, TNT can possibly polymerize and become completely insoluble (*3,7*). As the nitro groups become reduced to the amino form, they proceed through a hydroxylamine intermediate. It is thought that this unstable hydroxylamine can

form azoxy linkages at elevated pH values. Although some azoxy dimers are partially soluble in the aqueous phase and can be detected using HPLC, further polymerized molecules are completely insoluble. Since no azoxy dimers were detected using HPLC, polymerization was not suspected.

As expected, the $E_h$ was lowered by the oxidative utilization of starch by aerobic heterotrophs present in the bioslurry. $E_h$ values obtained in this demonstration were similar to those obtained in lab-scale treatability studies. Values fluctuated at times corresponding to the aggressive hydrolancing that may have introduced low levels of oxygen.

In a previous study, we have shown that TNT degradation can occur at a relatively wide temperature range (*3*). Since the WSOW demonstration was initiated in the autumn season, it was subjected to extreme temperature variations. The installation of the immersion heaters became necessary in order to maintain biological activity. Although the slurry temperature had dropped to as low as 4°C and had been heated to as high as 46°C, the degradation process persisted. This observation confirms the resilient nature of this bioremediation process. However, treatments should be performed above 20°C in order to maintain optimal conditions.

During the course of this demonstration, it was noted that the initial soil placed into the bioreactor was not completely mixed to homogeneity. Concentrations of TNT collected from five sampling sites were quite dissimilar and therefore caused large differences in each sample's level of contamination, as seen by the error bars on the graph of Fig. 4. Sample sites 1 and 2 started out with the highest concentrations of TNT and took the longest time to degrade. Although hydrolancing helped to mix the soil slurry, a more efficient method of mixing would have surely increased the rate of degradation.

At the arbitrary termination date, there were only very low levels of 24DA6NT found in the treated slurry. *p*-Cresol only transiently appeared throughout the degradation process. These compounds are also known intermediates in the anaerobic pathway for degradation of TNT (*8*). Similar results have been observed when an anaerobic bench-top reactor was fed a mixture containing TNT as the sole carbon source (*9*). If the reactor had been allowed to incubate a few weeks longer, and perhaps was augmented with additional starch, these intermediates would have likely also been reduced to nondetectable levels. The rejected rocks that were screened from the contaminated soil were hot-water-washed, and all waste water was treated in the process. However, it is possible that these rocks could still be contaminated and an extraction could be performed to determine if the rocks are actually clean. Data concerning the toxicity of the treated soil vs the untreated soil will soon be evaluated, but is not yet available.

It was shown by this demonstration that the bioremediation of TNT can be achieved using large-scale batches. This process may become extremely valuable for the treatment of a variety of nitroaromatic compounds that are resistant to the traditional aerobic process. It is predicted

that this bioremediation process will, therefore, be an economically competitive alternative to incineration for the waste management of TNT-contaminated soils.

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